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ORIGINAL ARTICLE

Prevalence, species distribution and antimicrobial resistance of enterococci isolated from dogs and cats in the United States

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Abstract

Aims: The contribution of dogs and cats as reservoirs of antimicrobial resistant enterococci remains largely undefined. This is increasingly important considering the possibility of transfer of bacteria from companion animals to the human host. In this study, dogs and cats from veterinary clinics were screened for the presence of enterococci.

Methods and Results: A total of 420 enterococci were isolated from nasal, teeth, rectal, belly and hindquarters sites of 155 dogs and 121 cats from three clinics in Athens, GA. Eighty per cent (124 out of 155) of the dogs and 60% (72 out of 121) of the cats were positive for enterococci. From the total number of dog samples ($n = 275$), 32% ($n = 87$) were from hindquarter, 31% ($n = 86$) were rectal, and 29% ($n = 79$) were from the belly area. The majority of isolates originated from rectal samples (53 out of 145; 37%) from cats. The predominant species identified was *Enterococcus faecalis* (105 out of 155; 68%) from dogs and *E. hirae* (63 out of 121; 52%) from cats. Significantly more *E. faecalis* were isolated from rectal samples than any other enterococcal species ($P < 0{\cdot}05$) for both dogs and cats suggesting site specific colonization of enterococcal species. The highest levels of resistance were to ciprofloxacin in *E. faecium* (9 out of 10; 90%), chloramphenicol resistance in *E. faecalis* (17 out of 20; 85%) and gentamicin resistance in *E. faecalis* (19 out of 24; 79%) from dog samples and nitrofurantoin resistance in *E. faecium* (15 out of 19; 79%) from cats. Multi-drug resistance (MDR) (resistance ≥2 antimicrobials) was observed to as few as two and as many as eight antimicrobials regardless of class.

Conclusion: This study demonstrated that dogs and cats are commonly colonized with antimicrobial resistant enterococci.

Significance and Impact of the Study: Dogs and cats may act as reservoirs of antimicrobial resistance genes that can be transferred from pets to people.

Introduction

Enterococci have been recovered from a number of habitats including the intestinal tract of mammals, soil, water, plants, insects, and food items (Witte *et al.* 1999; Giraffa 2002). They are a leading cause of nosocomial infections and are intrinsically more resistant to antimicrobial agents commonly used in hospitals than other bacteria (Martone 1998; Cetinkaya *et al.* 2000). In addition to being recognized as one of the primary causes of nosocomial infections, enterococci are also a reservoir of antimicrobial resistance genes (Landman and Quale 1997; Klare *et al.* 2001). The threat of untreatable enterococcal infections becomes more probable in light of increasing antimicrobial resistance, including multi-drug resistance (MDR). Further, the possible transfer of multiple-drug resistant determinants between bacteria complicates the problem (Murray 1998; Simjee *et al.* 2002; Guardabassi *et al.* 2004;

Leener *et al.* 2005). Food animals are typically an area of focus for study of the transfer of resistant zoonotic pathogens and commensals to humans. Much less attention has been centred on companion animals and their role in the persistence and dissemination of antimicrobial resistance to humans (Guardabassi *et al.* 2004).

Universally, companion animals, specifically dogs and cats in the American household, have evolved into the position of a close family member. According to the American Veterinary Medical Association (AVMA), more than 37% and 32% of American households own dogs and cats, respectively (http://www.avma.org/reference/marketstats/ownership.asp#companion). This equates to approx. 72 million dogs and 81 million cats with an average of 1·7 dogs and 2·2 cats per household of households that own pets. Dogs and cats have been previously recognized as sources of enterococci and may serve to harbour and transfer bacteria to humans due to the close physical contact that occurs between humans and their pets; the widespread use of antimicrobials in these animals increases the likelihood that these bacteria will also be resistant (van Belkum *et al.* 1996; De Graef *et al.* 2004; Guardabassi *et al.* 2004; Leener *et al.* 2005).

Few studies have examined healthy dogs and cats for the presence of enterococci and none of the studies has been performed in the US (De Graef *et al.* 2004; Leener *et al.* 2005; Moyaert *et al.* 2006; Delgado *et al.* 2007). Dogs and cats in American households average 2·6 and 1·7 veterinary clinic visits per year for various reasons (http://www.avma.org/reference/marketstats/ownership.asp#companion). In order to investigate the possible role of dogs and cats in carriage and potential dissemination of enterococci, this study determined prevalence and distribution of different species of enterococci from dogs and cats sampled at veterinary clinics in the Athens, GA, USA area. The antimicrobial susceptibility patterns associated with enterococci isolated from these animals were also evaluated.

Materials and methods

Sample collection, isolation, and identification of enterococci

During 2007, 155 dogs and 121 cats from three veterinary clinics in the Athens, GA, USA area were sampled. Samples from nasal, teeth, and rectal areas were collected using sterile swabs while sterile gauze was used for the belly and hindquarter areas. Swabs and gauze were premoistened with phosphate buffered saline (PBS, 1X), and kept refrigerated (4°C) until processed. Swabs and gauze were then transferred to 10 or 20 ml buffered peptone water (BPW, 1X), respectively, and incubated overnight at 37°C. One millilitre of each enrichment was then transferred to nine ml of Enterococcosel Broth (Becton Dickinson, Sparks, MD, USA) and incubated for 24 h at 37°C. Positive cultures were transferred to Enterococcosel Agar (Becton Dickinson) for isolation of enterococci. Plates were incubated overnight at 37°C. One presumptive positive colony was passed to blood agar, and the resulting clones were identified to enterococcal genus and species using multiplex PCR as previously described (Jackson *et al.* 2004).

Antimicrobial susceptibility

Minimum inhibitory concentrations (MIC, $\mu g\ ml^{-1}$) for enterococci were determined by broth microdilution using the Sensititre^{TM} semi-automated antimicrobial susceptibility system (Trek Diagnostic Systems, Inc., Cleveland, OH, USA) and the Sensititre^{TM} Gram-Positive Custom Plate CMV2AGPF according to the manufacturer's directions. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines when defined (CLSI, 2006, 2007). No CLSI interpretive criteria have been defined for flavomycin, kanamycin, lincomycin, and tylosin and only susceptible breakpoints have been established for daptomycin ($\leq 4\ \mu g\ ml^{-1}$) and tigecycline ($\leq 0{\cdot}25\ \mu g\ ml^{-1}$). Breakpoints for daptomycin, flavomycin, kanamycin, lincomycin, tigecycline, and tylosin were those defined by the National Antimicrobial Resistance Monitoring System (NARMS) (http://www.ars.usda.gov/Main/docs.htm?docid=6750&page=3). The panel of 17 antimicrobials and breakpoints for classification as resistant used by the NARMS program were as follows: chloramphenicol ($\geq 32\ \mu g\ ml^{-1}$), ciprofloxacin ($\geq 4\ \mu g\ ml^{-1}$), daptomycin ($\geq 8\ \mu g\ ml^{-1}$l), erythromycin ($\geq 8\ \mu g\ ml^{-1}$), flavomycin ($\geq 16\ \mu g\ ml^{-1}$), gentamicin ($\geq 500\ \mu g\ ml^{-1}$), kanamycin ($\geq 500\ \mu g\ ml^{-1}$), lincomycin ($\geq 4\ \mu g\ ml^{-1}$), linezolid ($\geq 8\ \mu g\ ml^{-1}$), nitrofurantoin ($\geq 128\ \mu g\ ml^{-1}$), penicillin ($\geq 16\ \mu g\ ml^{-1}$), streptomycin ($\geq 1000\ \mu g\ ml^{-1}$), quinupristin/dalfopristin ($\geq 4\ \mu g\ ml^{-1}$), tetracycline ($\geq 16\ \mu g\ ml^{-1}$), tigecycline ($\geq 0{\cdot}5\ \mu g\ ml^{-1}$), tylosin ($\geq 32\ \mu g\ ml^{-1}$), and vancomycin ($\geq 32\ \mu g\ ml^{-1}$). *Enterococcus faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were quality controls for determination of MIC.

Statistical analysis

Probability values of statistical significance were generated using chi-square analysis (SAS ver. 9.1.3; SAS Institute Inc., Cary, NC, USA). Statistical significance was defined as a probability value of less than or equal to 0·05 ($P \leq 0{\cdot}05$).

Table 1 Prevalence of enterococci from dogs and cats

					No. samples per isolation site (%)				
Animal	Reason for visit	No. animals tested	No. animals positive (%)	No. samples (*n* = 275)	Belly (*n* = 79)	Hindquarters (*n* = 87)	Nasal (*n* = 4)	Rectal (*n* = 86)	Teeth (*n* = 19)
Dog (*n* = 155)	Groom	35	28 (80)	58	12 (21)	14 (24)	1 (2)	23 (40)	8 (14)
	Boarding	66	52 (79)	115	35 (30)	40 (35)	1 (1)	32 (28)	7 (6)
	Patient	14	14 (100)	24	7 (29)	9 (4)	1 (4)	7 (29)	0
	Stray	4	4 (100)	23	7 (30)	7 (30)	1 (4)	6 (26)	2 (9)
	Resident	1	1 (100)	3	1 (33)	1 (33)	0	1 (33)	0
	Spay/Neuter	10	9 (90)	20	5 (25)	5 (25)	0	8 (40)	2 (10)
	Vaccine	14	10 (71)	20	8 (40)	7 (35)	0	5 (25)	0
	Bloodwork	1	1 (100)	2	1 (50)	1 (50)	0	0	0
	Dental	2	2 (100)	3	1 (33)	0	0	2 (66)	0
	Exam	8	3 (38)	7	2 (29)	3 (43)	0	2 (29)	0
				No. samples (*n* = 145)	Belly (*n* = 41)	Hindquarters (*n* = 36)	Nasal (*n* = 3)	Rectal (*n* = 53)	Teeth (*n* = 12)
Cat (*n* = 121)*	Groom	4	1 (25)	1	0	0	0	1 (100)	0
	Boarding	21	9 (43)	12	1 (8)	2 (17)	0	8 (67)	1 (8)
	Patient	17	13 (76)	28	8 (29)	6 (21)	2 (7)	9 (32)	3 (11)
	Stray	50	29 (58)	68	21 (31)	20 (29)	1 (1)	20 (29)	6 (9)
	Resident	7	7 (100)	19	9 (47)	5 (26)	0	4 (21)	1 (5)
	Spay/Neuter	14	12 (86)	15	1 (7)	3 (20)	0	10 (67)	1 (7)
	Dental	2	1 (50)	2	1 (50)	0	0	1 (50)	0

*Six cats submitted for vaccines were negative for enterococci and were omitted from the table.

Results

Prevalence of enterococci

A total of 275 samples from 155 dogs and 145 samples from 121 cats were collected (Table 1). These samples originated from animals visiting the veterinary clinics for various purposes including grooming, boarding, patient services (including surgery), spay/neuter, vaccines, bloodwork, dental exams, or regular physical exams; strays and residents at the clinics were also sampled. Of the animals, 80% (124 out of 155) of dogs and 60% (72 out of 121) of cats were positive for enterococci. The majority of dogs tested were those boarded at the clinics ($n = 66$) and 79% (52 out of 66) were culture positive for enterococci; nine of ten of the other categories of dogs also had a high percentage of positive animals ranging from 71% to 100% (Table 1). The only exception was dogs which were scheduled for exams and only 38% (3 out of 8) of those dogs tested positive for enterococci. In contrast, the majority of cats tested were strays ($n = 50$) of which 58% (29 out of 50) were positive for enterococci. The per cent positive for cats ranged from 25% (1 out of 4) for groomed cats to 100% (7 out of 7) for resident cats. Of the five areas tested for isolation of enterococci, the belly, hindquarters, and rectal areas yielded the highest numbers of enterococci while fewer isolates were obtained from the teeth and very low numbers were from the nasal area for both dogs and cats (Table 1). For all positive samples from dogs, 32% (87 out of 275) were from hindquarter, 31% (86 out of 275) were rectal, and 29% (79 out of 275) were from the belly area. Although less rectal samples were positive from cats (53 out of 121; 37%), the per cent positive was slightly higher than that from dogs (37% *vs* 31%) but not significantly different. The per cent positive per isolation site was not significantly different between dogs and cats (Table 1).

Identification and distribution of enterococci

Rectal, hindquarter, belly, teeth, and nasal samples collected from dogs and cats exhibited diversity in enterococcal species as the sites were positive for at least ten enterococcal species with three species, *E. faecalis*, *E. faecium*, and *E. hirae* isolated most frequently from both sets of animals (Table 2). The predominant species from dogs was *E. faecalis* ($n = 105$) while *E. hirae* ($n = 63$) was the most common species from cats. Some enterococcal species appeared to be preferentially associated with specific sites within the animals. For both dogs and cats, per isolation site, significantly more *E. faecalis* were isolated from rectal samples than from any other site ($P < 0{\cdot}05$). *Enterococcus faecalis* were isolated from 60% (52 out of 86) of rectal samples from dogs and 45% (24 out of 53) of rectal samples from cats (Table 2). Significantly more *E. faecalis* were also isolated from rectal samples than any other enterococcal species ($P < 0{\cdot}05$) for both dogs and cats. For dogs only, more *E. faecium* ($n = 26$) were also isolated from hindquarters than any other species. With the exception of nasal samples, this difference was not observed for *E. faecium* when isolation sites were compared against each other as almost equal numbers of *E. faecium* were isolated from the belly ($n = 22$) as from the hindquarters ($n = 26$) (Table 2). In cats, significantly more *E. hirae* were isolated from the belly than any other species, but no significant differences were observed between numbers of *E. hirae* from rectal ($n = 16$), hindquarters ($n = 17$), and belly ($n = 25$) samples compared against each other (Table 2).

Table 2 Distribution of *Enterococcus* species among dogs and cats

		No. species (%)*										
Animal	Site	*E. faecalis*	*E. faecium*	*E. hirae*	*E. casseliflavus*	*E. avium*	*E. gallinarum*	*E. mundtii*	*E. pallens*	*E. raffinosus*	*E. solitarius*	*E. species*
Dog	Rectal ($n = 86$)	52 (60)	7 (8)	13 (15)	1 (1)	6 (7)	1 (1)	0	0	0	0	6 (7)
	Hindquarters ($n = 86$)	23 (27)	26 (30)	20 (23)	11 (13)	0	0	1 (1)	0	0	0	5 (6)
	Belly ($n = 80$)	20 (25)	22 (28)	21 (26)	9 (11)	0	1 (1)	1 (1)	1 (1)	0	1 (1)	4 (5)
	Teeth ($n = 19$)	9 (47)	2 (11)	0	2 (11)	2 (11)	0	0	1 (5)	0	0	3 (16)
	Nasal ($n = 4$)	1 (17)	0	3 (50)	0	0	0	0	0	0	0	0
	Total ($n = 275$)	105 (38)	57 (21)	57 (21)	23 (8)	8 (3)	2 (0·7)	2 (0·7)	2 (0·7)	0	1 (0·4)	18 (7)
Cat	Rectal ($n = 53$)	24 (45)	4 (8)	16 (30)	0	7 (13)	1 (2)	0	0	1 (2)	0	0
	Hindquarters ($n = 36$)	7 (19)	11 (31)	17 (47)	0	1 (3)	0	0	0	0	0	0
	Belly ($n = 41$)	7 (17)	8 (20)	25 (61)	1 (2)	0	0	0	0	0	0	0
	Teeth ($n = 12$)	2 (17)	6 (50)	4 (33)	0	0	0	0	0	0	0	0
	Nasal ($n = 3$)	0	2 (67)	1 (33)	0	0	0	0	0	0	0	0
	Total ($n = 145$)	40 (28)	31 (21)	63 (43)	1 (0·7)	8 (6)	1 (0·7)	0	0	1 (0·7)	0	0

*Per cent species calculated by dividing number for each species by site.

Table 3 Antimicrobial resistance of enterococci isolated from dogs and cats

Antimicrobial*/ Animal	Breakpoint (μg ml^{-1})	No. of resistant (%)†								
		E. faecalis	*E. faecium*	*E. hirae*	*E. casseliflavus*	*E. avium*	*E. gallinarum*	*E. mundtii*	*E. solitarius*	*E. species*
Chloramphenicol (*n* = 20)	≥32									
Dogs		17 (85)								
Cats		2 (10)	1 (5)							
Ciprofloxacin (*n* = 10)	≥4									
Dogs			9 (90)							
Cats			1 (10)							
Erythromycin (*n* = 45)	≥8									
Dogs		23 (51)	8 (18)							
Cats		12 (27)	2 (4)							
Flavomycin (*n* = 228)	≥16									
Dogs		3 (1)	56 (25)	50 (22)	23 (10)	1 (0·4)	2 (0·9)	2 (0·9)	1 (0·4)	4 (2)
Cats			25 (11)	57 (25)	1 (0·4)	2 (0·9)	1 (0·4)			
Gentamicin (*n* = 24)	≥500									
Dogs		19 (79)								
Cats		5 (21)								
Kanamycin (*n* = 36)	≥500									
Dogs		19 (53)	6 (17)							
Cats		5 (14)	5 (14)			1 (3)				
Lincomycin (*n* = 357)	≥4									
Dogs		105 (29)	48 (13)	43 (12)	22 (6)	8 (2)	2 (0·6)	2 (0·6)		15 (4)
Cats		40 (11)	30 (8)	32 (9)	1 (0·3)	8 (2)	1 (0·3)			
Nitrofurantoin (*n* = 19)	≥128									
Dogs			4 (21)							
Cats			15 (79)							
Penicillin (*n* = 43)	≥16									
Dogs			27 (63)							
Cats			16 (37)							
Streptomycin (*n* = 40)	≥1000									
Dogs		4 (10)	20 (50)							
Cats		2 (5)	14 (35)							
Synercid (*n* = 3)‡	≥4									
Dogs			1 (33)		2 (67)					
Cats										
Tetracycline (*n* = 216)	≥16									
Dogs		42 (19)	42 (19)	47 (22)	2 (0·9)	3 (1)	2 (0·9)			
Cats		25 (12)	12 (6)	35 (16)		5 (2)	1 (0·5)			
Tylosin (*n* = 43)	≥32									
Dogs		23 (53)	6 (14)							
Cats		12 (28)	2 (5)							

*No isolates were resistant to daptomycin, linezolid or vancomycin.

†One *E. faecalis* isolate from a dog was resistant to tigecycline (breakpoint >0·5 μg ml^{-1}).

‡*Enterococcus faecalis* were not included in Synercid (quinupristin/dalfopristin) values.

Antimicrobial resistance

For eight of the antimicrobials tested (chloramphenicol, ciprofloxacin, erythromycin, gentamicin, nitrofurantoin, penicillin, streptomycin, and tylosin), antimicrobial resistance was restricted to two enterococcal species, *E. faecalis* and/or *E. faecium* (Table 3). In contrast, nine different enterococcal species were resistant to flavomycin, eight to lincomycin, and six to tetracycline. Of the 17 antimicrobials tested, *E. faecium* collectively was resistant to 12 different antimicrobials including chloramphenicol, ciprofloxacin, erythromycin, flavomycin, kanamycin, lincomycin, nitrofurantoin, penicillin, streptomycin, quinupristin/dalfopristin (Synercid), tetracycline, and tylosin. *Enterococcus faecalis* was resistant to ten antimicrobials overall excluding quinupristin/dalfopristin as *E. faecalis* are intrinsically resistant to this antimicrobial (Table 3). Other species ranged in number of resistances from one (*E. solitarius*) to four (*E. avium* and *E. casseliflavus*).

Table 4 Multidrug resistance patterns in enterococci from dogs and cats

Pattern*,†	No. of resistances	Species (no.)	No. animals	
			Dogs	Cats
ChlEryGenKanLinStrTetTyl	8	*Enterococcus faecalis* (1)	1	
ChlEryGenKanLinTetTigTyl	8	*E. faecalis* (1)	1	
ChlEryGenKanLinTetTyl	7	*E. faecalis* (15)	14	1
ChlEryLinTetTyl	5	*E. faecalis* (2)	1	1
ChlFlaKanLinNit	5	*E. faecium* (1)		1
CipEryFlaPenTet	5	*E. faecium* (1)	1	
EryFlaKanLinPenStrSynTyl	8	*E. faecium* (1)	1	
EryGenKanLinStrTetTyl	7	*E. faecalis* (5)	3	2
EryFlaKanLinPenStrTyl	7	*E. faecium* (4)	2	2
EryFlaLinPenStrTetTyl	7	*E. faecium* (3)	3	
EryGenKanLinTetTyl	6	*E. faecalis* (2)		2
EryFlaKanLinNit	5	*E. faecium* (1)	1	
FlaKanLinPenStrTet	6	*E. faecium* (1)	1	
FlaLinPenStrTet	5	*E. faecium* (25)	13	12
FlaLinTet‡	3	*E. hirae* (32)	29	3
		E. faecium (8)	8	
		E. gallinarum (3)	2	1
		E. faecalis (2)	2	
		E. casseliflavus (2)	2	
		E. avium (1)	1	
FlaLin‡	2	*E. hirae* (36)	9	27
		E. casseliflavus (19)	18	1
		E. faecium (5)	4	1
		E. species (4)	4	
		E. avium (2)		2
		E. mundtii (2)	2	

*Chl=chloramphenicol, Cip=ciprofloxacin, Ery=erythromycin, Fla=flavomycin, Gen=gentamicin, Kan=kanamycin, Lin=lincomycin, Nit=nitrofurantoin, Pen=penicillin, Str=streptomycin, Syn=Synercid (quinupristin/dalfopristin), Tet=tetracycline, Tyl=tylosin.

†Synercid was omitted from patterns where *E. faecalis* was the sole species exhibiting resistance.

‡Patterns with highest number of different enterococcal species.

The three antimicrobials for which the most diversity of species was observed (flavomycin, lincomycin, and tetracycline) were also the three with the highest levels of resistance. Eighty-five per cent of all isolates (357 out of 420) were resistant to lincomycin followed by 54% (228 out of 420) to flavomycin, and 51% (216 out of 420) to tetracycline (Table 3). Ten per cent or less of the isolates, collectively, were resistant to the other antimicrobials. By species, the highest levels of resistance was to ciprofloxacin in *E. faecium* (9 out of 19; 90%) followed by chloramphenicol resistance in *E. faecalis* (17 out of 20; 85%) and gentamicin resistance in *E. faecalis* (19 out of 24; 79%) all from dogs and nitrofurantoin resistance in *E. faecium* (15 out of 19; 79%) from cats (Table 3). By drug and species, the vast majority of resistance was below 50%; only nine instances of resistance exceeding 50% was observed for all drugs and all species. Only one *E. faecalis* isolate was resistant to tigecycline and none of the isolates were resistant to daptomycin, linezolid, or vancomycin (Table 3).

MDR defined in this study as resistance to two or more antimicrobials, was observed in the isolates and the patterns composed of five or more and those composed of the highest number of different enterococcal species are shown in Table 4. Isolates were resistant to as few as two and as many as eight antimicrobials. The largest groups of MDR based upon different patterns of antimicrobials belonged to those composed of three and four different antimicrobials (data not shown). Six different patterns for both three and four drug combinations were observed; the fewest different patterns was for the six drug combinations. Of the three isolates that were resistant to eight antimicrobials, two of the isolates were *E. faecalis* and one *E. faecium* all from dogs. The two eight drug MDR patterns exhibited by the *E. faecalis* isolates varied by two antimicrobials (streptomycin and tigecycline) with chloramphenicol, erythromycin, gentamicin, kanamycin, lincomycin, tetracycline, and tylosin common between the isolates (Table 4).

Two patterns (FlaLinTet and FlaLin) contained the highest number of different enterococcal species (Table 4). Both patterns contained six different enterococcal species with four of the same species (*E. hirae*, *E. faecium*, *E. avium*, and *E. casseliflavus*) common between the two patterns. *Enterococcus hirae* was the dominant species observed for both patterns. *Enterococcus hirae* with pattern FlaLinTet was found predominantly in dogs while *E. hirae* with pattern FlaLin was found predominantly in cats. The three antimicrobials (flavomycin, lincomycin and tetracycline) comprising these two patterns reflected the diversity in enterococcal species as many different enterococcal species were resistant to these drugs.

Discussion

Antimicrobial resistance in bacteria originating in companion animals is cause for concern. As a result of the close contact between companion animals and humans, the ease at which bacteria can be shared is magnified. Commensal bacteria such as enterococci have natural gene transfer mechanisms and can harbour multiple resistances; therefore, it is important to characterize the strains that are isolated from companion animals (Murray 1990). Other studies have investigated prevalence of enterococci from diseased or sick dogs and cats, but there is a paucity of data on enterococci isolated from healthy animals (De Graef *et al.* 2004; Leener *et al.* 2005). According to the latest market research statistics on US pet ownership, nearly half of all pet owners (49·7%) considered their pets as the equivalent of a family member (http://www.avma.org/reference/marketstats/sourcebook.asp) spending on average approximately $356 (US dollars) for dogs and $190 for cats per year per household for veterinary expenses. In this study, dogs and cats visited the veterinary clinics for a variety of reasons such as patient work, spaying or neutering, vaccines, bloodwork, and dental and physical exams as well as non-medical purposes such as grooming or boarding. Some of the animals were also residents of the clinics living there full-time or were strays being tended at the clinics. The variety of animals available and their different health status provided a unique opportunity to investigate the prevalence of enterococci and the antimicrobial resistance of the bacteria in animals that were not clinically ill.

The majority of dogs were positive for enterococci regardless of the reason the animal was in for a veterinary visit. The only exception was dogs which were subjected to physical exams. The total per cent positive samples from dogs including those in for physical exams was much higher than those reported previously for kennel or privately owned dogs (De Graef *et al.* 2004). The high traffic and continual introduction of animals in a clinic office may contribute to the higher prevalence. While previous studies on enterococci from cats have been performed, prevalence data was not readily available from those studies (Leener *et al.* 2005; Moyaert *et al.* 2006; Delgado *et al.* 2007). Therefore, these data serve as a benchmark and suggest that cats harbour less enterococci than dogs. This may be attributed to their relatively sheltered lifestyle and limited environmental contact outside of the house.

Enterococci were predominantly isolated from three sites on both dogs and cats: rectal, hindquarters, and belly. This observation was not unexpected since enterococci are a resident of the intestinal microflora and would likely contaminate the rectal, hindquarter, or belly area during or after defecation by the animal (Niemi and Ahtiainen 1995). This area is also in continual close contact with the environment than any other area. Although lower numbers of enterococci were isolated from the teeth and nasal areas, these areas did test positive. Taken together with the isolation of enterococci from the other areas, these data indicate that different areas of the animals can be contaminated with enterococci at any given time. This is especially important considering the close contact of companion animals with the human owners; the risk of transmission from the animals to the human host would be higher with contact to the rectal, hindquarter, or belly area, but could also occur with contact to the mouth or nose of the animals. Additionally, a positive body area could also contaminate the surrounding environment leaving open the potential for indirect spread of enterococci.

In most studies on enterococci from dogs and cats, five or fewer species of enterococci have been reported with *E. faecalis* and/or *E. faecium* isolated most frequently (Rodrigues *et al.* 2002; Poeta *et al.* 2006). *Enterococcus faecalis* and *E. faecium* are also the predominant species indicated in human infections (Murray 1990). In this study, at least ten different enterococcal species were isolated with *E. faecalis* as the predominant species isolated from dogs and *E. hirae* most frequently isolated from cats. This could be due to improved identification methods used in this study or the higher number of samples that were collected (Jackson *et al.* 2004). This is the first report of the preferential isolation of *E. faecalis* from rectal samples of dogs and cats. Interestingly, significantly more *E. hirae* were also isolated from belly samples than any other enterococcal species. Factors that influence the composition of the bacterial intestinal community have been described and may be resulting in the niche differences of *E. faecalis* and *E. hirae* observed in this study (Tannock 1999, 2005).

Resistance to lincomycin was high and was seen in all species of enterococci isolated except *E. solitarius*.

Intrinsic resistance to lincomycin has been described in previous studies (Murray 1990). High levels of cross-resistance to the macrolide, erythromycin, was not observed suggesting that the mechanism of resistance in the lincomycin resistant isolates was not due to target modification mediated by erythromycin resistance methylase (*erm*) genes (Roberts 2004). Levels of resistance to quinupristin/dalfopristin were also very low as only three isolates were resistant to these antimicrobials. *Enterococcus faecalis* isolates were not included in resistance levels for quinupristin/dalfopristin as this enterococcal species exhibits intrinsic resistance to the drug (Singh *et al.* 2002; Singh and Murray 2005). Some enterococci, particularly *E. faecium*, are inherently resistant to some penicillins; and in the past few years, they have also shown increased resistance to vancomycin, cephalosporins, and aminoglycosides in nosocomial infections (Fontana *et al.* 1990, 1996; Aarestrup *et al.* 1998). Vancomycin and quinupristin/dalfopristin are often considered the last treatment available in serious, multi-drug resistant, infections (Eliopoulos 2005). No resistance to vancomycin was found consistent with a number of studies on enterococci from dogs and cats (Rodrigues *et al.* 2002; De Graef *et al.* 2004; Leener *et al.* 2005; Delgado *et al.* 2007).

Resistance to tetracycline was high and observed in many of the different enterococcal species. Although determination of the genetic basis for resistance was not performed in this study, resistance to tetracycline in enterococci is most often mediated by *tet*(M) (Roberts 2005). This gene has been found in enterococci isolated from both dogs and cats in a previous study where 91% and 77%, respectively, of the *tet*M positive enterococci also contained a conjugative transposon (Leener *et al.* 2005). Tetracycline resistance was also common among isolates exhibiting MDR. Tetracyclines are used in dogs and cats for treatment of a variety of infections including urinary tract infections, periodontitis, upper respiratory tract infections and conjuctiva (Culver 1987; Kordick *et al.* 1997; Hayashi *et al.* 1998).

None of the isolates tested were resistant to the newer antimicrobials, daptomycin or linezolid although one *E. faecalis* isolate was resistant to tigecycline (MIC $\geq 0{\cdot}5$ μg ml^{-1}). Tigecycline is the first glycylcycline antimicrobial developed and acts by binding to the 30S ribosomal subunit in bacteria inhibiting protein translation (Bradford *et al.* 2005; Eliopoulos 2005). Tigecycline was designed to evade common resistance mechanisms exhibited by bacteria and is active against a number of bacteria including vancomycin-resistant enterococci (Bradford *et al.* 2005; Eliopoulos 2005). It was intended for skin, soft tissue and intra-abdominal infections and approved in 2005 for the treatment of Gram-positive and Gram-negative infections particularly Methicillin-Resistant *Staphylococcus aureus* (MRSA) and MDR *Acinetobacter baumannii*. Only a susceptible breakpoint ($\leq 0{\cdot}25$ μg ml^{-1}) has been established for tigecycline; however, the breakpoint of $\geq 0{\cdot}5$ μg ml^{-1} is presently used by the NARMS for the purpose of establishing a breakpoint for NARMS enterococcal isolates (http://www.ars.usda.gov/Main/docs.htm?docid=14491).

Of concern was the MDR patterns exhibited by the isolates. Three isolates were resistant to as many as eight antimicrobials; one *E. faecalis* with combined resistance to three aminoglycosides (gentamicin, kanamycin and streptomycin) and another *E. faecalis* with combined resistance to two aminoglycosides (gentamicin and kanamycin) and tigecycline. In addition, penicillin resistance was also present in some MDR *E. faecium* isolates. β-Lactam antimicrobials coupled with an aminoglycoside (gentamicin) or a glycopeptide is the usual treatment for enterococcal infections in humans (Wilson *et al.* 1995). Spread of the MDR enterococci from companion animals to humans could prove to be very difficult to treat in the human host.

The results from this study indicate that healthy dogs and cats are a source of antimicrobial resistant enterococci and may act as a reservoir of antimicrobial resistance that can be transferred from pets to people, people to pets, and within the environment. This risk is highlighted by antimicrobial resistance by use of the same antimicrobials used to treat infections in humans and pets. Furthermore, the enterococcal isolates were MDR exhibiting resistance to as many as eight antimicrobials. The extent of antimicrobial resistance in enterococci from healthy companion animals should be monitored to fully assess the role these animals have as reservoirs of resistant bacteria and their potential impact on humans. Additional studies will address the presence of antimicrobial resistance genes harboured by resistant isolates.

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